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14. ABSTRACT

Obesity is associated with increased risk and worse outcomes for ovarian cancer (OC). We theorize that the metabolic effects of obesity may play a contributing role in the pathogenesis of OC and lead to biologically different cancers than those that arise in normal weight women. We also posit that the timing and length of the obesity exposure may be critical in the development of obesity-driven OCs. We have demonstrated that adulthood exposure to obesity can promote tumor progression, as evidenced by a tripling in tumor size, in the KpB mouse model of serous OC. The ovarian tumors that arose in the obese mice were genomically and metabolically different from those that arose in lean mice. To expand on this work, we assessed *in utero*, adolescent and adulthood exposure to obesity as well as cross-over between these timeframes in the KpB mice. Obesity during adolescence and adulthood had a greater influence on tumor aggressiveness, as measured by increasing tumor size, than *in utero* exposure to obesity alone. Using The Cancer Genome Atlas database, we compared the gene expression between OCs from normal weight *versus* overweight/obese women. Metabolically relevant alterations in gene expression were found in relationship to BMI among serous OCs.

15. SUBJECT TERMS
ovarian cancer, obesity, mTOR pathway, genetically engineered mouse models, The Cancer Genome Atlas

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INTRODUCTION

Epithelial ovarian cancer is one of the most deadly cancers with an overall 5-year survival of only 30-40%. Increasing evidence suggests that obesity is a significant risk factor for ovarian cancer and is associated with worse outcomes for this disease (1-14). Less is known of the impact of the timing of the obesity exposure, but some epidemiological studies suggest that adolescent exposure to obesity bears the greatest increased risk for ovarian cancer development (12, 15). We hypothesize that the metabolic and endocrine effects of obesity play a role in the carcinogenesis of ovarian cancer and invariably lead to biologically distinct cancers than those that arise in leaner women, possibly through aberrant modulation of mTOR signaling in an obesity-specific mechanism. An understanding of the relationship between obesity across the lifespan of a woman, and mTOR activation in ovarian cancer pathogenesis has yet to be explored and makes this proposal novel. This translational proposal will address this gap in knowledge by investigating the impact of the timing of the obesity exposure *in vivo* and *in vitro* using a novel serous ovarian tumor murine model and in ovarian cancer tumors from obese and non-obese women via interrogation of The Cancer Genome Atlas (TCGA) Project database. We postulate that *in utero* and adolescent exposure to obesity will increase vulnerability to ovarian cancer, and this will be manifested in obesity-specific mTOR hyperactivation and its downstream effects on enhanced proliferation and an advantageous metabolic profile.

KEY WORDS
Ovarian cancer, obesity, mTOR pathway, genetically engineered mouse models, The Cancer Genome Atlas

Table 1. Diet-induced metabolic characteristics in non-obese and obese KpB mice.

	Lean	Obese	p-value
Weight (gms)	31.14 ± 5.26	50.71 ± 16.73	p=0.0003
Glucose (mg/dl)	186.81 ± 26.99	214.38 ± 58.11	p=0.053
% Fat	3.28 ± 1.51	19.58 ± 7.88	p=0.00001
% Lean	22.89 ± 2.11	28.66 ± 5.24	p=0.0006

N=14 mice per group. Mean ± SD. % Fat or % lean = each mass/total body mass as measured by MRI.

OVERALL PROJECT SUMMARY

Task 1 (Aim 1): To compare tumor latency and growth in K18-gT$_{121}^{+/-}$;p53$^{fl/fl}$;Brca1$^{fl/fl}$ (KpB) mice exposed to a high fat diet at different time points across the lifespan, including *in utero*, adolescence and adulthood.

Obesity and the KpB Mouse Model of Serous Ovarian Cancer. We have previously described a unique serous ovarian cancer mouse model that specifically and somatically deletes the tumor suppressor genes, Brca1 and p53, and inactivates the retinoblastoma (Rb) proteins in adult ovarian surface epithelial cells (KpB mouse model) (16, 17). Subsequently, KpB mice were subjected to a 60% calories-derived from fat in a high fat diet (HFD) *versus* 10% calories from fat in a low fat diet (LFD) to induce diet-induced obesity (N=14/group) starting at 6 weeks of age and until sacrifice. After 8 months of exposure to the HFD or LFD, obese mice weighed significantly greater than lean mice (p=0.003, **Table 1**). There was no effect of HFD on non-fasted blood glucose levels or diabetes onset in KpB mice over the course of the diet (**Table 1**). Body composition was significantly altered in obese KpB mice compared to lean controls. Percent body fat was six-fold greater in obese mice (**Table 1**, p=0.0001), while percent lean mass increased by 25% (p=0.0006, **Table 1**).

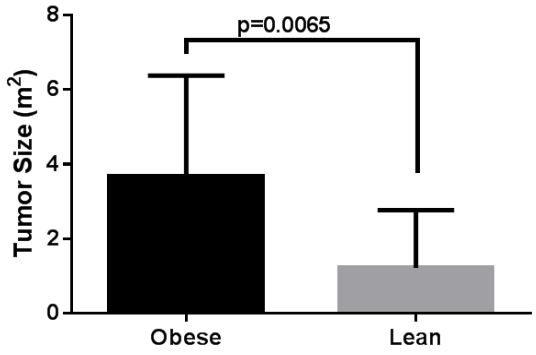

Figure 1. Obesity increases tumor size in KpB mice. KpB mice were fed low fat or high fat diets to induce obesity for 6 months during tumorigenesis. (A) Comparison of tumor size from lean and obese mice (N=14). These mice were sacrificed 6 months after ovarian tumor induction via injection of AdCre into the ovarian bursa cavity. For the calculation of tumor size, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined and multiplied (m^2). *p=0.0065.

The ovarian tumors were tripled in size in the obese mice as compared to lean mice (mean size of 3.7 cm^2 *versus* 1.2 cm^2, **Figure 1**, p=0.0065). This suggests that obesity can promote tumor progression in the KpB mouse model of ovarian cancer.

Genomic and metabolic differences characterize ovarian tumors arising in obese and lean mice. Gene expression and metabolomic profiling indicated statistically significant differences between ovarian tumors arising in obese *versus* lean mice (**Figure 2 & Table 2**) (18). 417 genes were up-regulated and 22 genes down-regulated in ovarian tumors from obese KpB mice *versus* lean mice (FDR<0.2), including genes involved in glucose, fatty acid and lipid metabolism as well as regulators of metabolic signaling pathways such as 5' adenosine monophosphate-activated protein kinase (AMPK). *Thus, the aggressive phenotype of OC in obese KpB mice was accompanied by upregulation of genes involved in metabolic and cell signaling pathways.*

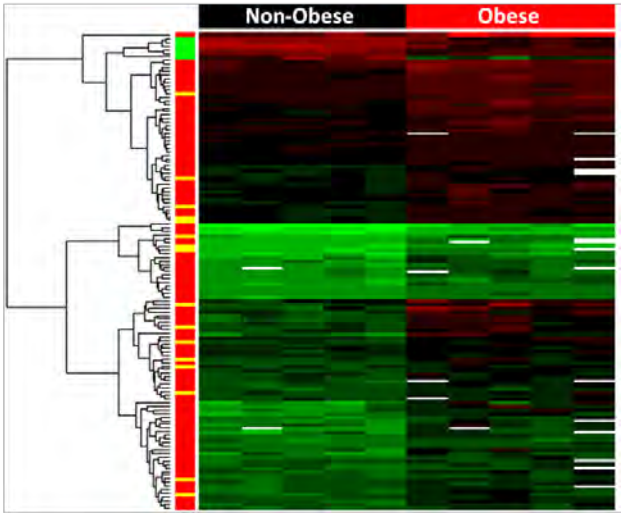

Figure 2. Genomic differences between ovarian tumors from obese *versus* lean KpB mice reveal alterations in metabolically relevant genes. Heat map representation of 131 genes significantly up- or down-regulated in ovarian tumors from obese *versus* lean KpB mice (FDR<0.1). Many metabolically relevant genes, such as lipocalin, fatty acid amide hydrolase, ectonucleoside triphosphate diphosphohydrolase, fatty acid 2-hydroxylase, glycerol-3-phosphate acyltransferase, protein phosphatase, protein kinase C and AMP deaminase 3, were upregulated in obese tumors.

Similarly, metabolomic profiling revealed metabolic differences between ovarian tumors from HFD-fed (obese) and LFD-fed (lean) KpB mice. 58 up- or down-regulated metabolites differentiated ovarian tumors in obese and lean mice. Glutamine (1.7 fold) and several fatty acids metabolites (5-10 fold) were found to be increased in the tumors from obese mice (p<0.05, **Table 2**). Most strikingly, glucose levels were 3-fold higher in the ovarian tumors of the obese *versus* lean mice (p<0.05), and were accompanied by decreases in downstream intermediates of glycolysis, including pyruvate and lactate, indicating impaired glycolysis (**Table 2**). This finding was unexpected because malignant cells generally increase glycolysis, preferentially metabolizing glucose to lactate for ATP production (the "Warburg" effect). As opposed to glucose, the more rapidly growing obese-OCs appeared to incompletely oxidize fatty acids for ATP production and fueling growth as opposed to glucose as evidenced by a 3-4 fold increase in several acyl-carnitines and dicarboxylic acids. Our gene expression profiling data support such a hypothesis as several lipases involved in lipid metabolism are upregulated in obese- *versus* lean-OCs; in addition, the AMPK gene was upregulated, and AMPK is involved in the activation of pathways that generate ATP such as fatty acid oxidation (19, 20) Lastly, succinate levels were almost 5-fold higher in the ovarian tumors from obese *versus* lean mice with a parallel decrease in fumarate and malate, indicating impaired succinate dehydrogenase (complex 2) activity. Aspartate, asparagine and glutamate feed into the tricarboxylic acid (TCA) cycle, and these were decreased in obese-OCs, further supporting a block at complex 2. Mitochondrial dysfunction has been reported in tumors cells (21), including that of complex 2 in OC (22). A schematic representation of

Sub-pathway	Biochemical Name	Obese/ Lean
Glycolysis, Gluconeogenesis and Pyruvate Metabolism	Glucose	2.76
	Fructose-6-phosphate	0.52
	Isobar: F1, 6BP, G1, 6BP, myo-INS BPs	0.45
	Pyruvate	0.48
	Lactate	0.76
TCA cycle	Succinate	4.84
	Fumarate	0.62
	Malate	0.71
Fatty Acid Oxidation	Palmitoylcarnitine	3.22
	Stearoylcarnitine	4.41
	Oleoylcarnitine	4.45
	Azelate	4.55
	Undecanedioate	4.48
Amino Acids	Aspartate	0.81
	Asparagine	0.73
	Glutamate	0.85

Table 2. Comparison of differences in glucose metabolism between the ovarian tumors from obese and lean KpB mice.

the metabolic alterations found in the ovarian tumors of the obese KpB mice can be found in **Figure 3**. *In summary, our findings suggest that obesity promotes changes in tumor genomics and metabolomics (i.e. an "obesity signature") that led to aggressive tumor behavior in the KpB OC mouse model.*

Effect of Obesity Exposures Across the Lifespan on Ovarian Tumor Progression To further expand on this work, the KpB ovarian cancer mouse model was used to assess the tumor promoting effect of high fat diet (HFD)-diet-induced obesity on tumor initiation and promotion amongst different *obesity exposures across the lifespan*. KpB mice were placed on a LFD or a HFD at different time points during their lifespan, including *in utero*, adolescence and adulthood. Cross-over diet study design was employed to examine if weight loss by switching from a HFD to a LFD reverses elevated risk associated with obesity. Eight different diet exposures were examined, as depicted in **Table 3**.

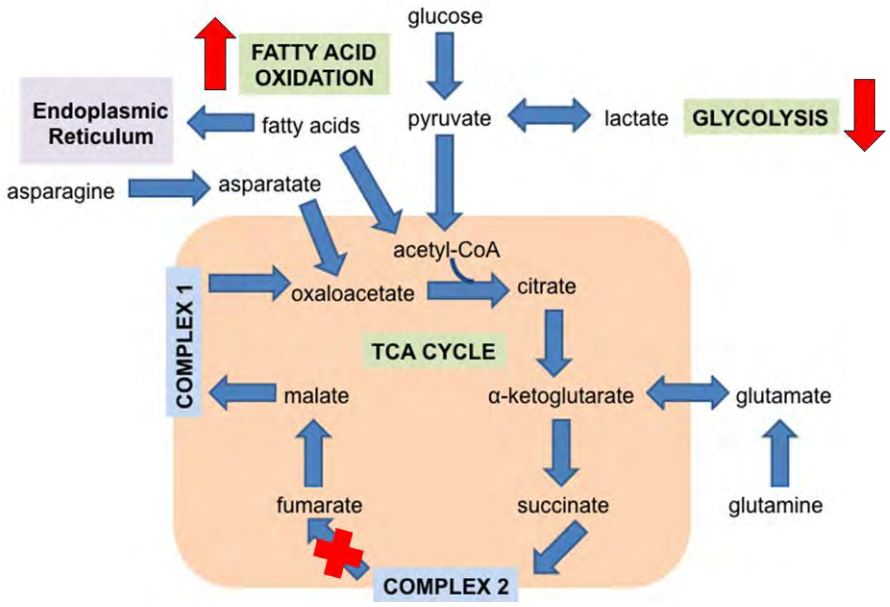

Figure 3. Summary of metabolic changes in ovarian tumors from obese *versus* lean KpB mice. The ovarian tumors from obese mice had evidence of increased fatty acid oxidation, decreased glycolysis and impaired mitochondrial 2 function as compared to the ovarian tumors from lean mice.

Table 3. Diet Study using the KpB ovarian cancer mouse model to identify relevant windows of susceptibility to obesity-induced risk. The effect of obesity on tumor promotion in mice made obese by high fat diet *versus* lean mice at relevant periods of vulnerability including *in utero*, adolescence and adulthood as well as combinations of these exposures were explored.

Group	*In utero* exposure	Adolescent exposure	Adulthood exposure
A	LFD	LFD	LFD
B	LFD	LFD	HFD
C	LFD	HFD	HFD
D	LFD	HFD	LFD
E	HFD	HFD	HFD
F	HFD	HFD	LFD
G	HFD	LFD	LFD
H	HFD	LFD	HFD

Tumor weight (grams) was found to be greater when comparing group A (no exposure to a HFD; LFD *in utero* + adolescence + adulthood) to groups B (LFD *in utero* + LFD in adolescence + HFD in adulthood), C (LFD *in utero* + HFD in adolescence + HFD in adulthood), D (HFD in adolescence + LFD *in utero* and adulthood), E (HFD *in utero* + HFD in adolescence + HFD in adulthood), F (HFD *in utero* and adolescence + LFD in adulthood) and H (HFD *in utero* + LFD in adolescence + HFD in adulthood) (**Figure 4**). The only group where tumor weight was not statistically different from group A (no exposure to a HFD; LFD *in utero* + adolescence + adulthood) was group G (HFD *in utero* + LFD in adolescence and adulthood). No differences were noted in tumor latency between any of these groups (data not shown). **This data does suggest that a HFD diet during adolescence and adulthood has a greater influence on tumor aggressiveness, as measured by increasing tumor size, than *in utero* exposure to obesity alone. However, the group with the largest tumors was group E, where the HFD was given during all 3 windows of exposure (i.e. *in utero*, adolescence and adulthood). Thus, longer exposure to a HFD resulted in the greater increase in tumor weight.**

The weight of the mice was also greater in the groups with longer exposure to the HFD. When compared to group A (LFD *in utero* + adolescence + adulthood), groups B (LFD *in utero* + LFD in adolescence + HFD in adulthood), C (LFD *in utero* + HFD in adolescence + HFD in adulthood), E (HFD *in utero* + HFD in adolescence + HFD in adulthood), G (HFD *in utero* + LFD in adolescence and adulthood) and H (HFD *in utero* + LFD in adolescence + HFD in adulthood) had a significant increase in body weight (**Figure 5**). Common to all the groups that had a statistically significant difference in mouse weight compared to group A (no exposure to a HFD) was adulthood exposure to a HFD, except for group G which represented *in utero* only exposure to obesity. The two groups that did not show a statistically significant increase in weight as compared to group A (no exposure to a HFD) were groups D (only adolescence exposure to obesity) and F (*in utero* and adolescence exposure to obesity). **Thus, in general, a switch in diet from a HFD *in utero*/adolescence to a LFD in adulthood resulted in a favorable decrease in weight gain. <u>However, despite this, ovarian tumor size was still increased in these groups.</u>** There were no significant changes in blood glucose levels among the eight groups (data not shown). The ovarian tumors from each of these groups are currently undergoing gene expression and metabolomic profiling comparative analysis.

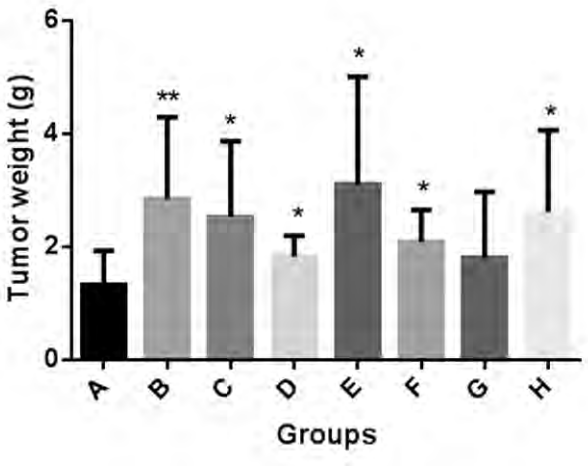

Figure 4. Exposure to a HFD increases the tumor weight of the KpB mice. KpB mice were placed on a LFD or a HFD at different time points during their lifespan, including *in utero*, adolescence and adulthood. (see **Table 3** for description of groups) (*p<0.05, **p<0.01)

Task 2 (Aim 2): To determine if PI3K/Akt/mTOR pathway hyperactivation and alterations in glucose metabolism are related to obesity-driven cancers in the KpB ovarian cancer mouse model.

Immunohistochemical analysis was performed of the ovarian tumors from the lean and obese KpB mice to assess proliferation, apoptosis, AMPK and downstream targets of the mTOR pathway **(Figure 6**). Ovarian tumors from obese and lean mice had similar expression of markers of proliferation (Ki-67); however, markers of apoptosis (caspase-3) were much increased in the ovarian tumors of obese *versus* lean mice (p=0.04). Expression of phosphorylated-AMPK was also increased in the setting of obesity (p=0.0235), corresponding to our genomics data in both mice and women. Lastly, expression of phosphorylated-S6, a downstream target of the mTOR pathway, was also slightly increased (p=0.0074), possible indicating increased mTOR pathway activation in ovarian tumors from obese mice.

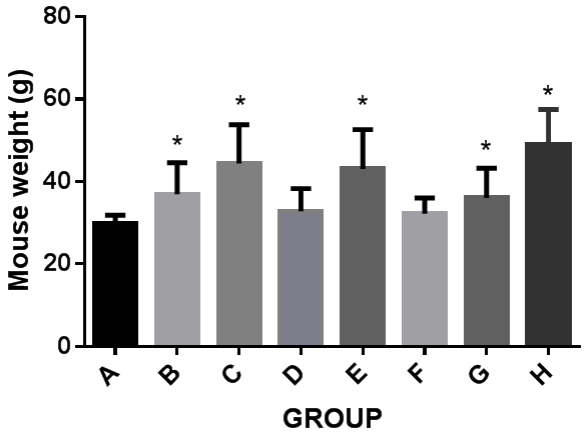

Figure 5. Exposure to a HFD increases the weight of the KpB mice. KpB mice were placed on a LFD or a HFD at different time points during their lifespan, including *in utero*, adolescence and adulthood. (see **Table 3** for description of groups) (*p<0.05)

Primary cultures were obtained from ovarian tumors from obese (M909) and lean (LM30) KpB mice, and these primary cultures (M909 and LM30) underwent studies to assess glucose uptake ([H^3]2-DG uptake assay) and glucose oxidation ([C^{14}]Glucose oxidation assay) (**Figure 7**). We found that glucose uptake (p=0.028) and glucose oxidation (p=0.0001) were increased in the LM30 compared to the M909 cells, consistent with our metabolomic data that glucose uptake and glycolysis was higher in ovarian tumors from lean *versus* obese mice. Studies to assess secretion of IGF-1, insulin, glucose, leptin and adiponectin are currently in progress. Future work will entail the assessment of fatty acid oxidation and mitochondrial respiration via cellular bioenergetic analysis.

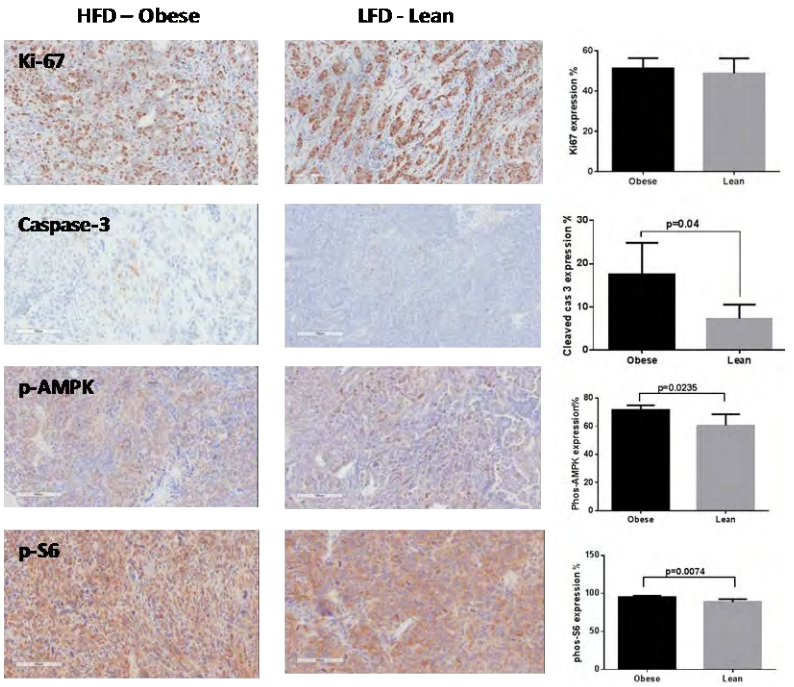

Figure 6. Immunohistochemical comparison between ovarian tumors from obese and lean KpB mice. Proliferative (Ki-67) and apoptotic (caspsae 3) markers were compared as well as phosphorylated-AMPK and phosphorylated–S6.

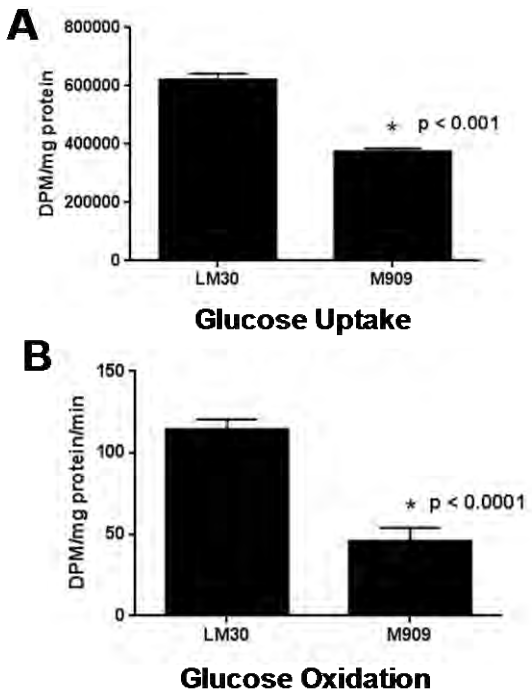

Figure 7. Comparison of glucose uptake and glucose oxidation in primary cultures or ovarian tumors from lean (LM30) and obese (M909) mice.

Task 3 (Aim 3): To assess cross-species differences of the gene expression profiles of ovarian cancer tumors from obese and non-obese women and KpB mice, using the relevant tumor data from The Cancer Genome Atlas (TCGA) Project database.

From the TCGA database, we collected expression measurements for 12,042 genes from the platform (BI_HT_HG-U133A level 3 data) for differential gene expression analysis among human serous OC samples. The detailed information of the data processing, quality control and normalization can be found on the TCGA website. To identify significantly differentially expressed genes associated with BMI, we

Table 4. Comparison of the demographics between the ovarian cancer tumors from normal weight and overweight/obese women.

	BMI < 25 (Normal Weight) (N=99)	BMI ≥ 25 (Overweight/Obese) (N=138)
Age (mean)	57.9	59.4
Race		
White	89 (90%)	125 (91%)
Black	5 (5%)	11 (8%)
Other	5 (5%)	2 (1%)
Grade		
2	11 (11%)	12 (9%)
3	88 (89%)	126 (91%)
Stage		
I/II	2 (2%)	4 (3%)
III/IV	97 (98%)	134 (97%)
Residual Disease		
Optimal	75 (76%)	99 (72%)
Suboptimal	24 (24%)	39 (28%)

applied linear modeling for responses as gene expression and covariates as 5 principal components (PCs) (from gene expression data to control potential batch effects), clinical stage, grade, age, race, residual tumor and BMI status (0 if normal BMI < 25; 1 if overweight BMI ≥ 25). Appropriate false discovery rates (FDR) were controlled. With the obtained genes that were significantly associated with BMI status, we conducted functional clustering analysis on the website of The Database for Annotation, Visualization and Integrated Discovery (DAVID). In addition, we applied hierarchical clustering analysis to generate a representative heatmap. The Chi-square test was used to compare BMI among different clusters of samples. A comparison of the demographics between the ovarian cancer tumors from normal weight (BM I< 25) and overweight/obese women (BMI ≥ 25) can be found in **Table 4**.

347 genes were found to be significantly up- or down-regulated with BMI status (BMI < 25 *versus* BMI ≥ 25) among the serous ovarian tumors (q-value < 0.1), including metabolically relevant genes related to fatty acid and lipid metabolism and transport as well as regulators of cellular metabolism (**Figure 8**), similar to our animal studies. Representative genes that were down-regulated included the prolactin receptor (3.6 fold) and apolipoprotein B mRNA editing enzyme (3.1 fold), among others. Representative genes that were up-regulated included mitogen-activated protein kinase 1 (3.3 fold), phospholipid scramblase 1 (3.3 fold), carnitine/acylcarnitine translocase (3.2 fold), low density lipoprotein receptor-related protein 8 (apolipoprotein e receptor) (3.7 fold), apolipoprotein L3 (3.7 fold), apolipoprotein L1 (3.8 fold), lipoyltransferase 1 (4.2 fold), apolipoprotein L6 (4.2 fold) and the c-myc binding protein (4.1 fold). Many of these genes were related to the apolipoprotein pathway, particularly apolipoprotein L related genes. Apolipoprotein L genes are members of the high density lipoprotein family and play a central role in cholesterol transport. Multiple genes involving the Ras oncogene family were up- and down-reulated when comparing normal weight *versus* overweight/obese women, including ras responsive element binding protein 1, RAB5C, RREB1, ras-related GTP binding C, PAP1A, RAB7A,

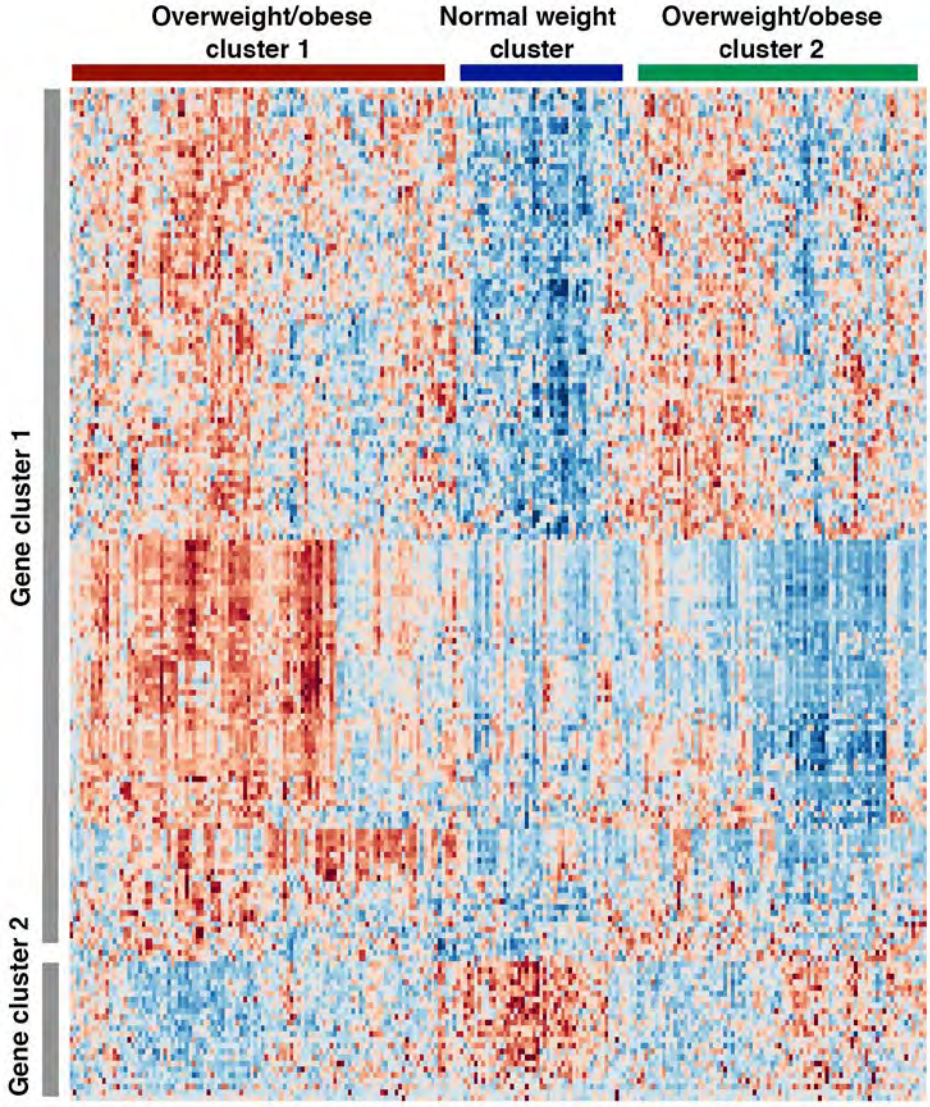

Figure 8. Genomic differences between ovarian tumors from normal weight *versus* overweight/obese women reveal alterations in metabolically relevant genes. Heat map representation of 264 genes significantly up- or down-regulated in ovarian tumors from normal weight *versus* overweight/obese women (FDR<0.05). Many metabolically relevant genes were upregulated in ovarian tumors from overweight/obese women such as mitogen-activated protein kinase 1, phospholipid scramblase 1, carnitine/acylcarnitine translocase, low density lipoprotein receptor-related protein 8 (apolipoprotein e receptor), apolipoprotein L3, apolipoprotein L1, lipoyltransferase 1, apolipoprotein L6 and the c-myc binding protein.

RAB31, RAB5A, and ras homolog family/member A. DAVID functional annotation analysis revealed significant enrichment in "protein transport" (Adjusted p-value for Benjamini = 5.5E-5), "antigen processing and presentation of exogenous peptide antigen" (Adjusted p-value for Benjamini = 1.3E-3) and "pyrimidine ribonucleotide biosynthetic process" (Adjusted p-value for Benjamini = 3.6E-2) for these identified genes.

	Gene Name	David Gene Name
Gene Cluster 1	FAP	fibroblast activation protein, alpha
	LAIR1	leukocyte-associated immunoglobulin-like receptor 1
	GPR65	G protein-coupled receptor 65
	RAB5C	RAB5C, member RAS oncogene family
	CTSK	cathepsin K
	RHOA	Ras homolog gene family, member A
	RAB5A	RAB5A, memver RAS oncogene family
	IL10RA	interleukin 10 recptor, alpha
	IL2RB	interluekin 2 receptor, beta
	LRP8	low density lipoprotein receptor-related protein 8
	APOL3	apolipoprotein L3
	APOL1	apolipoprotein L1
	CFLAR	CASP8 and FADD-like apoptosis regulator
	PLAU	plasminogen activator, urokinase
	RAB31	RAB31, member RAS oncogene family
	MYCBP	c-myc binding protein
	AAPOL 6	apolipoprotein L6
	LIPT1	lipoyltransferase 1
	PRKAA 1	protein kinase, AMP-activated, alpha 1 catalytic subunit
	PTPRC	protein tyrosine phosphotase, receptor type, C
	ETF1	eukaryotic translation termination factor 1
	EIF2B3	eukaryotic translational initiation factor 2B
	CASP1	caspase 1, apoptosis-related cysteine peptidase
Gene Cluster 2	IGSF3	immunoglobulin superfamily, member 3
	PRLR	prolactin receptor
	GRM4	glutamate receptor, metabotropic 4
	LY6G6E	lymphocyte antigen 6 complex, locus G6E
	GRIN1	glutamate receptor, ionotropic, N-methyl D-aspartate 1
	ADRA1 A	adrenergic, alpha-1A-, receptor

Table 5. Gene Clusters of the Ovarian Tumors from Normal Weight (BMI<25) and Overweight/Obese Women (BMI≥25).

Initially, we used the 347 genes with q-value < 0.1 to generate a heatmap, but the results of the hierarchical cluster analysis on these samples did not group them with a significantly different BMI distribution. Alternatively, we used the 175 genes with q-value < 0.05 to generate a heatmap, which is presented in **Figure 8**, where the row signifies gene expression and the column is clustering according to BMI (BMI < 25 *versus* BMI ≥ 25). If we specified two groups to cut a tree resulting from the results of the hierarchical cluster analysis on the samples, the two clusters of samples had no statistically significant difference in the distribution of BMI. However, if we specified three groups to cut a tree resulting from the results of the hierarchical cluster analysis, there were two pairs of clusters of samples with a significantly different distribution of BMI. Specifically, the first pair of clusters of samples (cluster 1 *versus* cluster 2) had sample proportions of subjects with BMI ≥ 25 (0.65,

0.33). For testing if the two proportions are significantly different, the obtained Chi-square statistics was 7.87, df = 1 and p-value = 0.005, suggesting that the two sample proportions are significantly different. The second pair of clusters of samples (cluster 3 *versus* cluster 2) had sample proportions of subjects with BMI ≥ 25 (0.61, 0.33). For testing if the two proportions are significantly different, the obtained Chi-square statistics was 11.36, df = 1 and p-value = 0.00075, suggesting that the two sample proportions are significantly different. In addition, there was significant difference in the proportions of women with a BMI ≥ 25 for cluster 1 and cluster 3 (0.65, 0.61). In summary, the analysis of the 175 gene set resulted in three sample clusters, with statistically significant differences in proportions of women with BMI ≥ 25 *versus* BMI <25 among these clusters. A summary of the genes in gene cluster 1 and 2 can be found in **Table 5**.

Cross-species comparisons between gene expression profiles of ovarian tumors from obese and non-obese women and mice revealed several upregulated genes in common, including AMPK. *Thus, mirroring our animal data, alterations in the expression of metabolically relevant genes in serous OC tumors were associated with elevated BMI in the TCGA database, with fatty acid and lipid metabolism and AMPK as potential common links between obese tumors in mice and women.*

KEY RESEARCH ACCOMPLISHMENTS

- The obese state can promote tumor progression in the KpB mouse model of ovarian cancer. Longer exposures to obesity had the greatest impact on tumor weight.
- Obesity during adolescence and adulthood had a greater influence on tumor aggressiveness, as measured by increasing tumor size, than *in utero* exposure to obesity alone.
- Overall, a switch in diet from a HFD *in utero*/adolescence to a LFD in adulthood resulted in a favorable decrease in weight gain. However, despite this, ovarian tumor size was still increased in these groups.
- Distinct metabolic and genomic differences were identified in ovarian tumors that arose in KpB mice after adulthood exposure to a HFD *versus* a LFD, and many of these differences were related to metabolic relevant pathways.
- The ovarian tumors from obese mice had evidence of increased fatty acid oxidation, decreased glycolysis and impaired mitochondrial 2 function as compared to the ovarian tumors from lean mice. Evidence for decreased glycolysis in ovarian tumors from obese *versus* lean KpB mice was confirmed in primary cultures.
- Increased expression of phosphorylated-AMPK and inhibition of the mTOR pathway (i.e. decreased phosphorylated-S6) was found in the ovarian tumors of obese *versus* lean KpB mice, along with higher levels of apoptosis.
- Metabolically relevant alterations in gene expression were found with increasing BMI among human serous ovarian cancers, using the TCGA database. Cross species comparisons found that fatty acid and lipid metabolism and AMPK as potential common links between obese tumors in mice and women.

CONCLUSION

Obesity is associated with increased risk and worse outcomes for OC, and alterations in PI3K/Akt/mTOR signaling may play a crucial role in this relationship with potential implications for prevention and improvement of outcomes for this disease. We and others have made significant progress investigating the effects of obesity on tumor cell growth, but an understanding of the interactions between obesity at specific vulnerable periods of development, OC and the PI3K/Akt/mTOR pathway is lacking. We theorize that the metabolic effects of obesity play a contributing role in the pathogenesis of OC and lead to phenotypically different cancers than those that arise in leaner women, potentially through hyperactivation of the mTOR kinase. We also posit that the timing and length of the obesity exposure is critical in the development of obesity-driven OCs. Although the PI3K/Akt/mTOR pathway is known to be separately important in obesity and OC, the complex interplay between both of these disease entities on PI3k/Akt/mTOR signaling in OC has not been simultaneously assessed. Our multi-dimensional approach is innovative because utilizes several tools including a unique genetically engineered mouse model (GEMM), cell lines and patient samples to comprehensively interrogate the obesity-induced carcinogenesis signature through molecular, biochemical, genomic and metabolomic analysis. While the PI3K/AKT/mTOR pathway is a likely candidate pathway, the defined approach will identify additional genes and metabolites heretofore underappreciated in OC that are dependent upon windows of obesity exposure.

To first study the connection between metabolic status and OC, we capitalized upon the novel K18-$gT_{121}^{+/-}$;$p53^{fl/fl}$;$Brca1^{fl/fl}$ (KpB) OC genetically engineered mouse model (GEMM) in the obesity-susceptible FVB/N background. KpB mice were used to investigate the impact of obesity on OC pathogenesis and to test the effects of varying the timing and length of the obesity exposure across the lifespan, including *in utero*, adolescence and adulthood. Adulthood exposure to obesity in the KpB mouse model accelerated tumorigenesis. A tripling of tumor mass was found in KpB mice fed a high fat obesogenic diet *versus* a low fat control diet in our initial pilot study. Gene expression and metabolomic profiling indicated statistically significant differences between the ovarian tumors from the obese *versus* lean mice, including metabolically relevant pathways. This included a 3-fold increase in glucose levels and energy supplied by fatty acid oxidation rather than glycolysis as well as impairment of mitochondrial complex 2 activity. Increased expression of phosphorylated-AMPK and inhibition of the mTOR pathway (i.e. decreased phosphorylated-S6) was found in the ovarian tumors of obese *versus* lean KpB mice, along with higher levels of apoptosis.

We expanded on this work to assess which windows of exposure to obesity are most important for increased vulnerability and progression of OC. Eight different diet exposures were examined, as depicted in **Table 3**. Longer exposure to a HFD resulted in greater tumor weight. Obesity during adolescence and adulthood had a greater influence on tumor aggressiveness, as measured by increasing tumor size, than *in utero* exposure to obesity alone.The weight of the mice was also greater in the groups with adulthood exposure to a HFD. In addition, a switch in diet from a HFD *in utero*/adolescence to a LFD in adulthood resulted in a favorable decrease in weight gain. However, despite this, ovarian tumor size was still increased in these groups. The ovarian tumors from each of these eight groups are currently undergoing gene expression profiling and metabolomic comparative analysis

This proposal aims next to translate *in vitro* and preclinical studies into human relevance and explore the impact of obesity on human OC. Using The Cancer Genome Atlas (TCGA) database, we compared the gene expression between OCs from normal weight *versus* overweight/obese women. Metabolically relevant alterations in gene expression were found in relationship to BMI status among serous OCs, including multiple genes related to the apolipoprotein pathway. Apolipoproteins are proteins that bind lipids (oil-soluble substances such as fat and cholesterol) to form lipoproteins for transport through the lymphatic and circulatory systems. This will be pathway of interest for our future studies. Cross species comparisons found that fatty acid and lipid metabolism and AMPK as potential common links between obese tumors in mice and women, enabling us to identify obesity-dependent biomarkers and potential novel targets of treatment for future study. Cross-species comparisons lends strength to findings from either strategy, thus creating a powerful experimental paradigm. The proposed study design is innovative because the tools we used such as the KpB mouse model and the TCGA database allows us to focus on the tumor/host interaction, especially in regards to obesity-driven effects. As part of our future studies, we will collect ovarian tumors from obese and lean women for metabolomic profiling analysis. Through these complementary studies, we will advance the understanding of OC's metabolic and genomic responses to the tumor promoting environment of obesity and determine the impact of the timing and length of the obesity exposure on ovarian carcinogenesis.

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Publications:

(1) Makowski, L, Zhou, C, Zhong, Y, Kuan, PF, Fan, Sampey, BP, Difurio, M and Bae-Jump, VL. Obesity increases tumor aggressiveness in a genetically engineered mouse model of serous ovarian cancer. Gynecologic Oncology, 133(1):90-7 (2014). PMID: 24680597

Abstracts presented:

(1) **Bae-Jump V**, Zhou C, Zhong Y, Du X, Makowski L, Jia W. Diet-induced obesity increases tumor aggressiveness in a genetically engineered mouse model of serous ovarian cancer. 44th Annual Meeting of the Society of Gynecologic Oncology, March 2013, Los Angeles, California.

(2) Wysham, WZ, Chen, TH, Makowski, L, Mutch, D, Berchuck, A, Karlan, B, Levine, DA, **Bae-Jump, VL**, Relationship between Body Mass Index (BMI) and gene expression profiles of high grade serous ovarian cancers in The Cancer Genome Atlas (TCGA) project, 29th Annual Meeting of the Mid-Atlantic Gynecologic Oncology Society, October 2014, Chapel Hill, North Carolina.

(3) Wysham, WZ, Zhang, Y, Dickens, HK, Malloy, KM, Han, XY, Guo, H, Gehrig, PA, Zhou CX, **Bae-Jump VL**. Differential efficacy of metformin *versus* everolimus in the setting of obesity in a mouse model of serous ovarian cancer. 106th Annual Meeting of the American Association for Cancer Research, April 2015, Philadelphia, Pennsylvania.

(4) Wysham, WZ, Chen, TH, Makowski, L, Levine, D, Mutch, D, Berchuck, A, Karlan, B, **Bae-Jump, VL**, Relationship between obesity and gene expression profiles of high grade serous ovarian cancers in The Cancer Genome Atlas (TCGA) project, 46th Annual Meeting of the Society of Gynecologic Oncology, March 2015, Chicago, Illinois.

INVENTIONS, PATENTS AND LICENSES

None

REPORTABLE OUTCOMES

None

OTHER ACHIEVEMENTS

Grants submitted:

American Cancer Society
Research Scholar Grant – RSG CCE 128826
Obesity, Cation-Selective Transporters and Metformin in Endometrial Cancer
January 2016 – December 2019
$659,626 (total direct costs), $164,963 (annual direct cost for year one)
Principal Investigator: Bae-Jump
20% effort (2.4 calendar)
Mounting epidemiological and preclinical data suggest that metformin may be efficacious in endometrial cancer. However, two important questions that need to be addressed are: (1) Will metformin be universally effective in endometrial cancer or be more efficacious in the obese/insulin-resistant patient population? and (2) What role do transporters play in metformin uptake and action in the malignant endometrium? These fundamental questions will be explored in endometrial cancer, a disease driven by obesity and insulin resistance, using endometrial cancer mouse models and phase 0 and phase 2/3 clinical trials in endometrial cancer patients.
NOTE: Scored "outstanding" and within the payline for ACS, awaiting council review.

NIH/NCI - 1R01CA204859-01
Obesity-Induced Metabolic Signature of Ovarian Cancer and Impact on Treatment
April 2016 – March 2021
$1,250,000 (total direct costs), $239,170 (annual direct cost for year one)
Principal Investigator: Bae-Jump
25% effort (3.0 calendar)
Ovarian cancer arising in obese mice is more aggressive and is characterized by a genetic and metabolic profile distinct from tumors developing in lean mice, resulting in increased sensitivity to the anti-tumorigenic effects of metformin. We hypothesize that these obesity-driven alterations are initially established by the nutrient-rich obese host environment and become fixed such that subsequent changes in the metabolic host

milieu become irrelevant. This will be explored through (1) delineating a timeline of genetic and metabolomic divergence between ovarian tumors in obese and lean mice using our unique KpB mouse model, (2) *via* transplantation of ovarian tumors arising in either obese or lean environments to the opposite host phenotype and assessing for metformin responsivity and (3) through an ongoing phase 2 trial of metformin/paclitaxel/carboplatin in ovarian cancer patients.
NOTE: This grant is to be reviewed on October 5th, 2015.

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DOD/OCRP: Obesity Exposure Across the Lifespan on Ovarian Cancer Pathogenesis

PARTICIPANTS

I. PERSONNEL

This DOD/OCRP Pilot Award application is synergistic, requiring and capitalizing on the skills of multidisciplinary participants. Dr. Bae-Jump is a gynecologic oncologist and translational cancer researcher with extensive experience in the investigation of targeted therapies in ovarian and endometrial cancer. Dr. Bae-Jump will serve as Principal Investigator for this project. She has assembled an experienced team of outstanding researchers and collaborators, creating a unique environment to study the impact of timing and length of the obesity exposure on ovarian cancer pathogenesis. Co-investigator Dr. Makowski has expertise and training in obesity research, metabolism, and inflammation. Dr. Damania has an impressive track record in cancer biology, signal transduction, and virology and will also serve as a Co-Investigator for this project. By bringing together experienced researchers and collaborators with diverse and complementary skills, we will increase our efficiency to ensure our collective success. The work proposed would be a new project for our laboratories and cannot be done without the support of DOD funds.

Victoria Bae-Jump, M.D., Ph.D., Principal Investigator, (2.4 cal months, 20% effort, no salary requested). Dr. Bae-Jump is an Assistant Professor in the Division of Gynecologic Oncology. She is a gynecologic oncologist and a translational cancer researcher at the Lineberger Comprehensive Cancer Center (LCCC) in the Clinical Research Program. Dr. Bae-Jump received her PhD and MD and did a postdoctoral fellowship at Virginia Commonwealth University. She performed her residency and fellowship at UNC Chapel Hill in Obstetrics & Gynecology and Gynecologic Oncology. Dr. Bae-Jump joined the faculty at UNC in July 2007 and was awarded an institutional Multidisciplinary Clinical Research Career Development Roadmap K12 grant. In September of 2010, she subsequently received a 5 year NIH/NCI K23 Mentored Patient-Oriented Research Career Development Award. Her research focuses on understanding the interactions between cell signaling pathways implicated in endometrial and ovarian cancer pathogenesis as a means to target therapy for this disease. In this pursuit, she has investigated many novel targeted therapies for the treatment of endometrial and ovarian cancer, including mTOR inhibitors, arsenic, a human monoclonal antibody to the insulin growth factor-1 receptor (IGF-1R), soy, genistein and most recently, metformin. In addition, she has a clinical trial underway that is a preoperative window study of metformin in obese endometrial cancer patients. She has also expanded her interests to include the development of an ovarian cancer mouse model that will be utilized in this project. Dr. Bae-Jump has expertise in molecular and cellular biology, animal and cell culture studies as well as microarray and immunohistochemical analysis for the exploration of alterations in cell signaling pathways in gynecologic malignancies. Dr. Bae-Jump will oversee the experimental design, implementation and analysis of this project and will directly interact with her research associate, Dr. Chunxiao Zhou as well as Cheng Fan, a Bioinformatics Statistician. **Dr. Bae-Jump is funded 75% by her K23 grant that was awarded in September of 2010 and 25% by her clinical activities. Thus, she does not request any additional salary support. Dr. Bae-Jump will commit 20% of her time to this project.**

Liza Makowski Hayes (a.k.a. Liza Makowski), M.M., Ph.D., Co-Investigator (2.4 cal months, 20% effort). Dr. Makowski is an Assistant Professor of Nutrition, a member of the Nutrition Obesity Research Center, Center for Gastrointestinal Biology and Disease, McAllister Heart Center, and Lineberger Comprehensive Cancer Center at UNC Chapel Hill. Dr. Makowski's Ph.D. in the Department of Nutrition at the Harvard School of Public Health with Dr. Gökhan Hotamisligil focused on how fatty acid transporters modulate the macrophage inflammatory response and cholesterol metabolism in atherosclerosis, for which she received NRSA and NIH-LRP fellowships. She also obtained a Masters in Medicine as a Lucille P. Markey Fellow from Harvard Medical School during her Ph.D. studies. Her postdoctoral studies under Drs. Deborah Muoio and Chris Newgard in the Departments of Medicine and Pharmacology & Cancer Biology at Duke University addressed the role of mitochondrial fuel metabolism in times of overnutrition using animal and cell culture models in combination with comprehensive metabolomic profiling at the Duke Stedman Center for Nutrition and Metabolism. She

currently holds a K99/R00 to investigate the role of macrophage lipid metabolism and inflammation in obesity, and a U01 to examine the effect of post-partum obesity on breast cancer focusing on the inflammatory microenvironment. She has expertise in mitochondrial metabolism, inflammation, microarray and metabolomic analysis, and histological analysis in animal and cell culture-based obesity, cancer, and diabetes/insulin resistance research. Dr, Makowski and Damania have a manuscript in progress on metabolomics and fuel metabolism in virally induced lymphoma. Dr. Makowski will supervise Dr. Freemerman in the design of all experiments, review and guide the interpretation of all data, and aid in preparation of the manuscript. **Dr. Makowski will commit 20% of her time to this project.**

Blossom Damania, PhD, Co-Investigator (0.36 cal months, 3% effort). Dr. Damania is a Professor in the Department of Microbiology and Immunology and a member of the Lineberger Comprehensive Cancer Center at UNC Chapel Hill. She established her lab in October 2000 and has been continuously funded both through NIH and private foundations. Dr. Damania has been studying the PI3K/Akt/mTOR pathway and its impact on oncogenesis for the last ten years and has published nine papers related to this pathway. In addition, she has developed and tested multiple anti-cancer compounds in tumor xenograft mouse models which were developed and validated by her and are available for this project (i.e NVP-BEZ235). Her laboratory has identified inhibitors of the upstream kinases in the PI3K/Akt/mTOR pathway that prevent tumor growth in pre-clinical studies, and Dr. Damania is currently involved in the initiation of clinical trials at UNC Hospitals to test inhibitors of PI3K and mTOR on all viral and non-viral Non-Hodgkin lymphomas. She hopes to extend these targeted agents to the treatment of ovarian cancer. Dr. Makowski and Damania have ongoing collaboration and manuscript in process documenting the role of glucose versus fatty acid metabolism in B cell lymphomas. Dr. Damania will collaborate on the experimental design and interpretation of the cell culture and animal studies. **Dr. Damania will commit 3% of her time to this project.**

Alex J. Freemerman, Ph.D., Research Associate/Lab Manager (6 cal months, 50% effort). Dr. Freemerman is the laboratory manager and lead research associate for Dr. Makowski's lab. He holds a Ph.D. in Cell Biology and has more than 15 years of experience in bench research focused on pre-clinical cancer drug discovery, including 10 years as a Research Assistant Professor in the Department of Surgery at Duke University Medical Center studying xenografts in mouse models. Dr. Freemerman has expertise in examining macrophage, cancer cell, and adipocyte-derived stem cell signaling cascades, metabolic biochemical assays, as well as considerable experience in managing rodent colonies, diet-induced obesity studies in mouse models, culturing primary cells, minor rodent surgery, and immunohistochemistry. **Dr. Freemerman will commit 50% of his time to this project.**

Chunxiao Zhou, M.D., Ph.D., Research Associate (6 cal months, 50% effort, no salary support requested). Dr. Zhou is the laboratory manager and lead research associate for Dr. Bae-Jump's lab. Dr. Zhou has a M.D., Ph.D. awarded in China. He joined the Division of Gynecologic Oncology at UNC-CH in 2000 as a postdoctoral research associate and has worked in the laboratory of Dr. Bae-Jump since July 2007. He has a vast range of experience with molecular and cellular techniques, including *in vitro* gene expression studies, PCR, electrophoresis, cell-based assays, SDS-PAGE, Western immunoblotting, immunhistochemical techniques as well as animal/mouse work. **He will contribute 50% of his time to this project, but no salary support is requested. Dr. Zhou's salary is funded by the Department of Obstetrics and Gynecology.**

Cheng Fan, MS, Bioinformatics/Statistician (0.24 cal months, 2% effort, no salary requested). Cheng Fan, MS (UNC-CH) from the Genomics/Bioinformatics Core Facility will serve as the Bioinformatics/Statistician for this work. He has a Masters Degree in both Computer Information Science and Computational Biology, and joined the Lineberger Comprehensive Cancer Center in 2004 as a Bioinformatics Research Associate. Cheng Fan has extensive experience in analyzing DNA microarray expression data for a variety of cancers, most notably breast cancer. He has developed statistical strategies for analyzing microarray datasets associated with a multitude of cancers and has provided consultations for many researchers in experimental design and data analysis in regards to gene expression profiling and data mining. Cheng Fan will assist with the comparison of the gene expression profiles of the ovarian cancer tumors from the obese and non-obese women in the TCGA

ovarian cancer project, as well as the cross-species comparisons with the KpB mouse model. **Cheng Fan's salary is provided by Lineberger Comprehensive Cancer Center, and thus, salary support is not requested for his contribution to this project.**

COLLABORATORS

D. Neil Hayes, M.D., Ph.D., Collaborator (0 cal months, 0% effort). Dr. Hayes is an Associate Professor of Hematology Oncology and is an expert on clinical and translational genomics of aerodigestive tumors. At UNC, he has generated genomic and array data on over 1000 patients, including gene expression arrays, SNP chips, methylation profiles, tissue microarrays, and DNA sequencing. Techniques employed include genomic profiling, epigenetic analysis, targeted sequencing, and high-resolution shotgun sequencing. He has participated in method development for genomic analysis including hypothesis testing, high-throughput sequence analysis, and quality assessment and has designed and executed numerous clinical cancer therapy trials. Dr. Hayes is the Clinical Director of Bioinformatics for the Lineberger Comprehensive Cancer Center, with primary oversight for their clinical and genomic data systems and is the Medical Director of the UNC Hospitals Tumor Registry. Dr. Hayes also serves as Co-PI for UNC's participation in the Cancer Genome Atlas (TCGA), a pivotal project of the National Institutes of Health. Dr. Hayes and Dr. Makowski have collaborated previously on the role of a DNA repair enzyme in head and neck cancer patients, and wrote a review on LKB1 and AMPK in lung cancer. Dr. Hayes will work with Dr. Bae-Jump and her lab for the optimization of the tissue microarrays and immunohistochemical protocols that are to correlate expression of key components of the glucose metabolism pathway and the closely related IGF-1R and PI3K/Akt/mTOR pathways with ovarian tumors derived for obese versus lean women and mice. **No salary support requested.**

Charles Perou, Ph.D., Collaborator (0 cal months, 0% effort). Dr. Perou is an Associate Professor of Genetics and Pathology and Laboratory Medicine and is Scientific Director of the UNC-CH Genomics and Bioinformatics Core Facility and Co-Director of the Mouse Phase I Unit (MPIU). He is a leading figure in cancer gene expression profiling, and is Co-Director of the The Cancer Genome Atlas (TCGA) project at UNC-CH. UNC-CH was one of twelve centers chosen for this large-scale, collaborative effort by the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI). Their goal is to systematically characterize the genomic changes that occur in various cancers. The focus of Dr. Perou's laboratory is the characterization of the biological diversity of human tumors using genomics, molecular genetics, and cell biology. He is directly involved in the translation of these technologies to develop improved diagnostics and therapies that are specific for each cancer subtype. As further testament to his expertise, Dr. Perou won the 2009 American Association for Cancer Research (AACR) Outstanding Investigator Award in Breast Cancer for his work on deciphering the underlying biology of distinct molecular subtypes of breast cancer. Dr. Perou and Dr. Bae-Jump have ongoing collaborations in the microarray analysis of both ovarian and endometrial cancer tumors. Dr. Perou also collaborates with Dr. Makowski on her U01 breast cancer-obesity grant. Dr. Perou's role will be to assist Dr. Bae-Jump in the analysis of the microarray gene expression data derived from ovarian cancer specimens from obese and non-obese women and KpB mice, especially in regards to the cross-species comparisons. He will help Dr. Bae-Jump in the supervision of Cheng Fan, a Bioinformatics Statistician. In addition, Dr. Perou will provide the resources and expertise of the Mouse Phase I Unit for the implementation of the studies on the timing and length of the obesity exposure in the KpB ovarian cancer mouse model. **No salary support requested.**

Wei Jia, Ph.D., Co-Director, Collaborator, NORC Metabolomics Core (0 cal months, 0% effort). Dr. Wei Jia is a Professor in the Department of Nutrition and the Director of the UNC-Greensboro Center for Research Excellence in Bioactive Food Components at our Kannapolis Campus. He also serves as the Co-Core Director for the NORC Metabolomics Core. Dr. Jia is an expert in metabolomic studies and profiling and his research interests lay in the area of identifying metabolite markers for disease, studying baseline metabolic signatures to predict how individuals will respond to a specific nutritional intervention and, in addition, understanding how bioactive phytochemical compounds interact with metabolic pathways. As Director of the UNCG Center for

Research Excellence in Bioactive Food Components, Dr. Jia is responsible for assisting researchers with human studies requiring metabolic profiling. He directs a state-of-the-art facility that includes a metabolomic profiling platform featuring an Agilent HPLC TOF-MS system and Leco GC-TOF-MS system. Dr. Jia provides metabolite expression patterns in both human and animal samples for NORC users. Dr. Jia will supervise the metabolomics data collection and analysis and be actively involved in the elucidation of biochemical pathway perturbations involved in obesity and ovarian cancer pathogenesis in the KpB mouse model. **No salary support requested.**

Xiuxia Du, PhD, Collaborator, Bioinformatics Concierge, NORC Metabolomics Core (0 cal months, 0% effort). Dr. Du is an Assistant Professor of Bioinformatics at UNC-Charlotte, and the Bioinformatics Concierge for the NORC Metabolomics Core. She received her Ph.D. in Systems Science and Mathematics from Washington University in St. Louis in 2005. She subsequently did her post doctorate research in Dr. Richard D. Smith's lab at the Pacific Northwest National Laboratory. Her research is focused on developing bioinformatic approaches for integrating metabolomic and nutrigenomic data analyses. Dr. Du works with NORC members who are conducting systems biology nutrition research helping them to use and develop bioinformatic tools in analyzing this complex data. Her office is located at our Nutrition Research Institute (NRI) Kannapolis campus. She will assist with the metabolomics data analysis and be actively involved in the elucidation of biochemical pathway perturbations involved in obesity and ovarian cancer pathogenesis in the KpB mouse model. **No salary support requested.**